Application No. 09/747,521
Amendment dated September 9, 2003 (Revised November 6, 2003)
Reply to Office action of May 7, 2003
Docket Number 22727/04079

Amendments to the Specification (Amendments made with reference to the original specification as filed.)

Please replace the paragraph on page 4 that begins with line ~~19~~ 21 and runs through line 23, with the following amended paragraph:

FIG. 3 shows the Plasmid pCI (Promega Inc.), the eukaryotic expression vector which was used to express ~~aa~~ amino acids 9-252 of the amino acid sequence shown in Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2, of *B. anthracis* lethal factor protein, and ~~aa~~ amino acids 175-735 of the amino acid sequence shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4, of *B. anthracis* protective antigen protein.

Please replace the paragraph on page 5 that begins with line 18 and runs through page 6, line 4, with the following amended paragraph:

In one aspect, the immunogenic composition comprises a protein or polypeptide which comprises the B. anthracis lethal factor protein, preferably a mutated form of the lethal factor protein such as LF7, which contains a single amino acid substitution of a glutamic acid for a ~~cepteine~~ ~~redidue~~ cysteine residue at position 687 of the amino acid sequence shown in Figure 1B, i.e., amino acid 720 of SEQ ID NO. 2, or an immunogenic fragment thereof. As used herein the term "immunogenic fragment" refers to a peptide which is at least 6 amino acids in length, preferably at least 15 amino acids in length, and has the ability to elicit production of antibodies that bind to the wild-type protein from which it was derived, in this case the LF protein. The LF protein may be a full-length, wild-type, mature LF protein. The full-length, wild-type, mature LF protein has a molecular weight of 90 kDa and comprises 764 amino acids. In one embodiment, the full-length, wild-type, mature LF protein comprises the amino acid sequence, SEQ ID NO: 2, shown if FIG. 1. The term "LF protein", as used herein, also encompasses naturally-occurring and mutated LF proteins whose sequence differs from the sequence shown in FIG. 1. Such variant proteins have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "LF protein reference sequence" shown in FIG. 1. Such variant proteins have an altered sequence in which one or more of the amino acids in the LF protein reference sequence is substituted, or in which one or more

amino acids are deleted from or added to such sequence. Such variants, when injected into an animal, elicit production of antibodies that bind to the mature, wild-type LF protein, i.e., the LF protein whose sequence is depicted in FIG. 1.

Please replace the paragraph on page 6 that begins with line 16 and runs through line 22, with the following amended paragraph:

Variant sequences, which are at least 90% identical, have no more than 1 alteration, i.e., any combination of deletions, additions or substitutions, per 10 amino acids of the flanking amino acid sequence. Percent identity is determined by comparing the amino acid sequence of the variant with the reference sequence using MEGALIGN module in the DNA STAR program. One example of a suitable variant of the LF protein shown in FIG. 1 is the LF7 protein which except for a substitution of a glutamic acid for a cysteine at amino acid position 687 of the amino acid sequence shown in Figure 1B, i.e., amino acid 720 of SEQ ID NO. 2, has a sequence which is identical to the LF protein reference sequence.

Please replace the paragraph on page 9 that begins with line 19 and runs through page 10, line 1, with the following amended paragraph:

In another aspect, the present invention relates to nucleic-acid based immunogenic compositions which comprise a polynucleotide which encodes the B. anthracis LF protein or, preferably, a mutated form of the LF protein , referred to hereinafter as the "LF polynucleotide", or an immunogenic fragment thereof, referred to hereinafter as the "LF fragment polynucleotide" and methods of using such immunogenic compositions. The LF polynucleotide may encode a full-length mature LF protein or, preferably, a mutated LF protein such as LF7. In one embodiment, the LF fragment polynucleotide comprises nucleotide ~~125~~ 124 through nucleotide 855 of the sequence, SEQ ID NO. 1, shown in Figure 1. In another embodiment, the LF polynucleotide comprises nucleotide 100 through 2430 of SEQ ID NO. 1. In one embodiment, the LF fragment polynucleotide comprises nucleotide 125 through nucleotide 855 of the sequence, SEQ ID NO. 1, shown in FIG. 1. The LF polynucleotide or LF fragment polynucleotide is operably linked to a promoter which drives expression of the protein or fragment. Such promoter may be a constitutive promoter, such as for example the viral promter derived from cyomegalovirus

(CMV) Alternatively, the promoter may be an inducible promoter such as, for example, the lac promoter or a tissue specific promoter, such as the whey acidic protein promoter.

Please replace the paragraph on page 13 that begins with line 2 and runs through line 10, with the following amended paragraph:

The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the LF protein. The plasmid construct pCLF4 encodes the LF protein fragment consisting of amino acids 9-252 of the amino acid sequence shown in Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2, which includes the PA binding site. This plasmid was constructed from a PCR-amplified fragment using the primers 5'-CTGAAACCATCACGTAAAA-3', SEQ ID NO. 5, and 3'-AGCAAGAAATAAATCTATAGTCTAGA-5', SEQ ID NO. 6, which contain Xba cut sites. The Xba-digested PCR and pCI plasmid fragments were ligated to form the pCLF4 plasmid used in these studies. The resulting plasmid construct pCLF4 does not contain a signal sequence for secretion of the expressed gene product. All plasmids were purified from E. coli DH5α using the Endo-free plasmid preparation kits (Qiagen) and resuspended in PBS before use.

Please replace the paragraph on page 13 that begins with line 12 and runs through line 16, with the following amended paragraph:

Protein preparations. The LF and LF7 antigens used in these studies were expressed and purified as previously described (Leppla 1988; Park 2000. Optimized production and purification of Bacillus anthracis lethal factor. Prot. Exp. Purif. 18:293-302). LF7 is the full-length LF protein which contains a mutation at position 687 (E687C) of the amino acid sequence shown in Figure 1B, i.e., amino acid 720 of SEQ ID NO. 2, in the zinc-binding active site thus eliminating the metalloproteinase activity of LF.

Please replace the paragraph on page 15 that begins with line 17 and runs through line 25, with the following amended paragraph:

The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the LF protein. The gene fragment encoding amino acids

175-735 of the amino acid sequence shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4, of the PA protein was PCR amplified using the plus strand primer (5'-CTCGAGACCATGGTT-3', SEQ ID NO. 7) and minus strand primer (3'-TAAGGTAATTCTAGA-5', SEQ ID NO. 8) using pYS2 as a template (Welkos 1988; Singh 1994). Included in the primer sequences are Xho and Xba restriction cut sites, respectively. The PA gene fragment expressed in these studies represents the PA_{63} protease-cleaved fragment of the full-length 83 kDa protein that is active in vivo (Gordon 1995). The PCR reaction product was digested with XhoI and Xba and ligated into the pCI vector which had been cut with the same two restriction enzymes..

Please replace the paragraph on page 16 that begins with line 5 and runs through line 17, with the following amended paragraph:

These examples utilized the pCI mammalian expression vector (Promega) which utilizes the human cytomegalovirus (CMV) immediate-early enhancer-promoter region for strong, constitutive expression of the incorporated gene (FIG. 3). Use of this expression vector results in high level expression of a non-secreted form of the encoded gene product. In these examples we chose to express only partial sequences of the PA and LF genes as shown in FIG. 3. The pCPA plasmid expresses a truncated version of the PA gene product (aa 175-735 of the amino acid sequence shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4) which is the PA_{63} antigen lacking the furin cleavage site (aa 164-167) yet is fully functional in vivo (Gordon 1995. Proteolytic activation of bacterial toxins by eukaryotic cells is performed by furin and by additional cellular proteases, Infect. Immun. 63:82-87.). The pCLF4 plasmid expresses a truncated form of LF (aa 9-252 of the amino acid sequence shown in Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2) which lacks the catalytic domain of LF, yet retains PA_{63} binding activity and is therefore capable of interacting with the truncated form of PA expressed from pCPA (Arora, Klimpel et al. 1992. Fusions of anthrax toxin lethal factor to the ADP-ribosylation domain of Pseudomonas exotoxin A are potent cytotoxins which are translocated to the cytosol of mammalian cells. J Biol Chem 267(22):15542-8.).... ....

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Please replace the paragraph on page 19 that begins with line 12 and runs through line 15, with

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the following amended paragraph:

Example 3. Inducing a Protective Immune Response Against Challenge with B. anthracis ~~Sores~~ Spores by a Prime Boost Method Which Employs a DNA Plasmid Encoding an Immunogenic Fragment of LF, a DNA Plasmid Encoding an Immunogenic Fragment of PA, and a Booster Immunization with Purified rPA/rLF7